Migration on Variable Moduli Substrates

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Abstract

No effective treatments exist for metastatic breast cancer, producing a 5-year survival rate of only 20%[1-3]. Current *in vitro* migration models lack both the fibrous nanostructure of native tissue and engineered variations in modulus preventing accurate and rapid development of new treatment modalities[5-9]. Electrospun nanofibers on polydimethylsiloxane (PDMS) substrates provide a biomimetic platform allowing evaluation of cellular migration while simultaneously tailoring substrate mechanics and topography [7]. In this study, substrates with moduli ranging from that of natural breast tissue (18-244 kPa)[4] to that of relatively hard (1610 kPa) PDMS were tested to demonstrate how cell migration on nanofiber changes with the modulus of the underlying substrate. The MDA-MB-231 breast cancer cell line was inoculated on these scaffolds and their migration potential assessed using time-lapse microscopy. Additionally, Scanning Electron Microscopy (SEM) was used to determine the substrate-fiber interface and the cell morphology along these substrates. The results show a total nanofiber-based migration of 238 μm on ‘stiff’ versus 108 μm on ‘soft’ substrates.

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1. Introduction and Objectives

Metastatic breast cancer is responsible for 90% of breast cancer-related deaths. Currently, there is no effective cure for the disease and the 5-year survival rate is only 22%(1-3). It has repeatedly been shown that as a tumor progresses it is influenced by internal genetic changes as well as external environment cues. Often cancer progression is discussed as a series of genetic and epigenetic changes that affect the cell's ability to grow, recruit supporting cells, and eventually metastasize. Recently it has been acknowledged that the surrounding microenvironment plays a vital role in influencing a tumor cell's migration, proliferation, and overall viability. The extent to which a tumor's microenvironment encourages or discourages metastasis is a growing area of interest in the cancer field. It has been suggested that rearrangement of the extracellular matrix proteins is one factor which plays a vital role in metastasis. The Extracellular Matrix (ECM) is typically composed of collagen, and elastin. It had been shown that as a tumor progresses the Collagen becomes rearranged in an aligned manner. This alignment helps to facilitate migration away from the original tumor site. The mechanical properties of a cell's microenvironment have also been shown to affect a broad range of the cell behavior including cell morphology, matrix remodeling, differentiation, cell survival, and metabolism. It has also been shown that cell motility is affected by substrate stiffness (10). These results indicate that cells are sensitive to changes in the mechanical properties of the surrounding microenvironment. It has also been noted that a change in the mechanics of the microenvironment also encourages metastasis. As a tumor progresses the secretion of Lysol oxidase from the surrounding stromal cells crosslinks the ECM proteins increasing the stiffness of the tumor microenvironment (11). Though the influence of these factors on migration has been well established in literature there exists a lacking in the *in vitro* substrates available to test migration while modeling the topography and modulus of the vivo environment. The development of effective, personalized anti-invasive diagnostic and therapeutic approaches has been impeded largely due to a lack of appropriate *in vitro* models (5-9). These essays lack both the topography and the mechanical effects of an *in vivo* environment and as such the migration of the cell occurs in a different way. Cellular migration *in vivo* occurs along collagen or elastin fibers. The fibers allows the clusters of integrin complexes from a cell's surface to act in a stretching and retracting motion in order to facilitate movement along the fiber. In contrast migration along plastic as in a scratch assay on flat plastic does not promote integrin clustering

and as such migration often occurs by very different mechanisms. This study will evaluate how changes in substrate modulus and topography effect a cell's migration. This study will also investigate a novel nanofiber-polymer integrated platform adaptable to high-throughput anti-invasive drug screening. To develop better treatment methods for metastatic breast cancer a better biomimetic platform is needed (7). The developed platform will focus on two aspects of the breast cancer extracellular matrix (ECM): the modulus of the surrounding tissue and its nanofibrous structure. The results have clinical relevance as they allow for the development of aligned nanofibrous scaffolds having an engineered underlying substrate to allow for tests of future cancer treatments. The developed model may also provide a clinical diagnostic tool allowing for the assessment of tumor biopsies and confirming the presence of highly migratory cancer cells.

2. Approach and Experimental Procedures

2.1 Scaffold Formation

Polydimethylsiloxane (PDMS) scaffolds were fabricated using blends of Sylgard 184 and Sylgard 527 polymer. The blends were formed by first mixing the respective cross linker and polymer together and then mixing the two different polymer-crosslinker solutions together to form the desired ratios. These ratios were: 100% 527, 80% 184 and 20% 527, 50% 184 and 50% 527, 20% 184 and 80% 527, and 10% 184 and 90% 527. The total amount of PDMS used was 3ml. The mixed polymer was heated at 67°C for 12-24 hours to ensure the most uniform cure in the samples (10). The PDMS scaffolds will serve as a model for a change in the modulus experienced *in vivo* to model the topography of the *in vivo* environment. Polycaprolactone (PCL) dissolved in HFP (5 wt%) was electrospun onto the PDMS scaffolds with a flow rate of 2 ml/hr for 45 minutes onto a rotating wheel to provide alignment to the scaffolds. All scaffolds were plasma treated for two minutes and then sterilized for 24 hours under UV light.

2.1 Mechanical Testing

The modulus of the various polymer blends were tested using a tensile test. The strain rate was 5 mm/minute. The modulus was calculated by fitting a line in the first ten percent of the stress strain curve.

2.2 Migration Testing

In order to test the migrational potential on the variable substrates time-lapse microscopy was used. First the scaffolds were treated with DMEM F-12 cell culture media for 24 hours. Then MDA-MB-231 GFP labeled cells were seeded on the each platform at a density of 20,000 cells per well. The cells were allowed to adhere for 24 hours. Using time-lapse microscopy the cells were then imaged every half hour for 24 hours. The images were analyzed using imageJ software to determine the total migration distances, net migration distance, and velocity. The results were analyzed using minitab software to determine the statistical significance.

2.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to understand how the morphology of the cell changed on the various substrates and to understand if the fiber-PDMS interface changed as the modulus of the PDMS decreased. In order to image the fiber-PDMS interface samples were mounted and sputter coated to allow for SEM imaging. In order to image the cell morphology on the various moduli fibers cells were inoculated onto the scaffolds at 20,000 cells/well and allowed to adhere for 24 hours. Cells were then washed with PDMS and fixed using Gluteraldehyde. After fixing cells were dehydrated, mounted, and sputter coated to allow for SEM imaging.

3. Results and Discussion

3.1 Modulus Determination

The created PDMS ratios were tested in tension to determine how the modulus of the underlying scaffold affected the cells. The results of this test can be seen in Table 1 below.

Table 1: Modulus determination of various PDMS blends

Material (Percent 527)	**Experimental Modulus (kPa)**	**Std.**
0	1610	334
20	1014	16.733
50	862	29.5
80	180	5.6
90	46	8.9

As can be seen from the above table the lowest modulus of PDMS was 54 kPa. This substrate is a good model for native noncancerous breast tissue which has a modulus of 57 kPa. An infiltrating ductal tumor can be represented by the 50% 527 and the 80% 527 samples as an infiltrating ductal tumor has a modulus of 450 kPa (Reference).

The modulus of the PDMS was tested as the factor which affects migrations because fibrous substrates have already been tested and it has been shown that changes in the fiber modulus do not affect cell migration (12). It is acknowledged that the modulus of the PDMS is not the modulus that the cell directly feels and that some interaction between the cell and the PDMS occurs; however, the assumption is made that modulus "felt" by the cell is similar to that of the PDMS.

3.2 Cell Migration

After formation of the PDMS-nanofibrous scaffolds time-lapse microscopy was used to determine the total distance migrated of MDA-MB-231 cells. The results can be seen in figure 1 below.

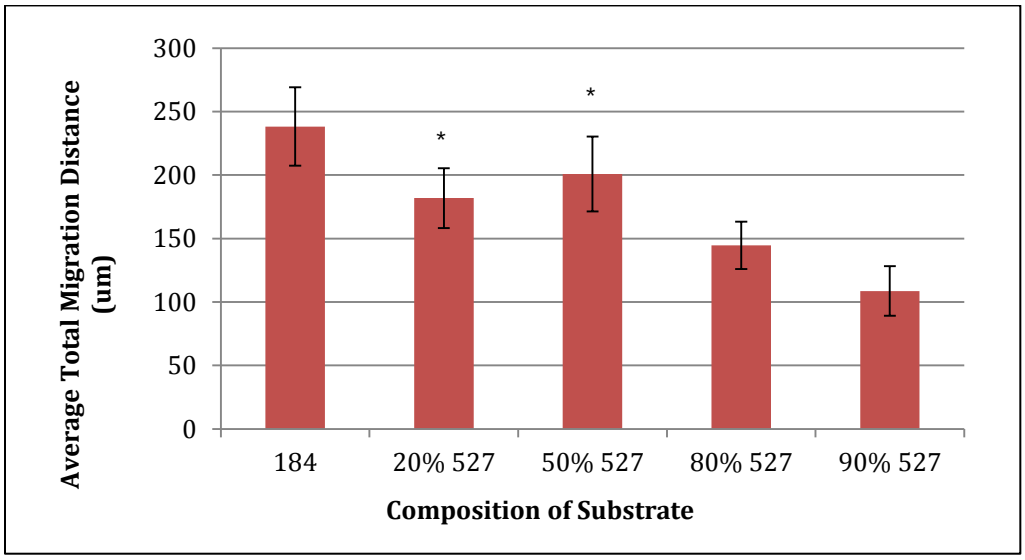

Figure 1: Average total distance migrated by MDA-MB-231 cells on PDMS-nanofibrous scaffold.

The above figure shows that better migration can be seen on the harder materials while the softer materials do not allow for as great of migration. These results suggest that adding a modulus

aspect into standard migration tests would vastly change migrational results. The above chart also shows a flip in the number 20% 527 and the 50% 527 data however the difference in these two values are not statistically significant so the trend holds that a harder substrate gives a greater migration. These results can also be viewed in terms of the velocity and the net migration of cells. Velocity and Net migration can be seen in figure 2 and 3 respectively.

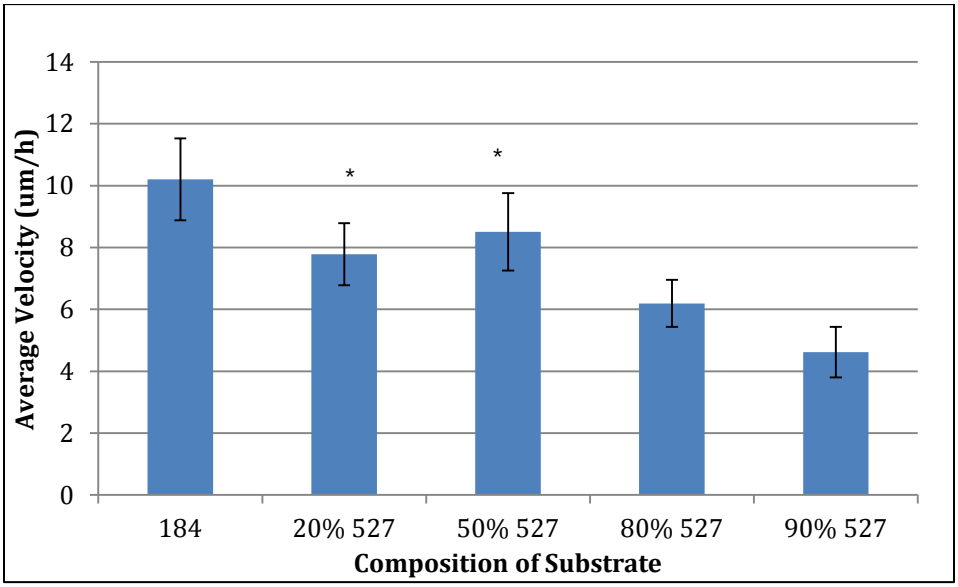

Figure 2: Velocity of the MDA-MB-231 cells on PDMS-Nanofibrous scaffolds.

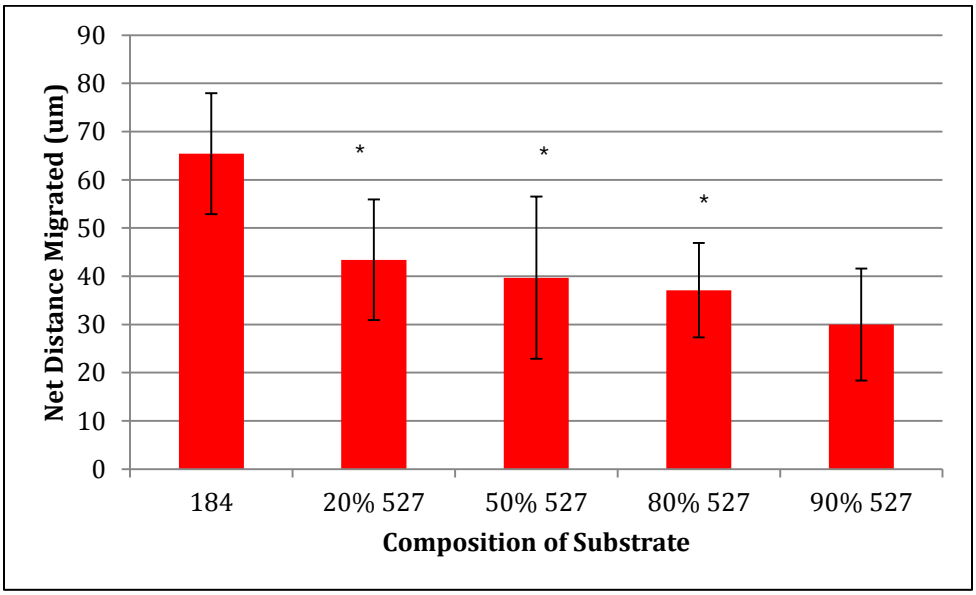

Figure 3: Net Distance Migrated by the MDA-MB-231 cells on PDMS-Nanofibrous scaffolds

The above graph of velocity is important to analyze as it allows easy comparisons to other migration studies which have been run for various amounts of time. Net Migration is important because we can determine if a cell is migrating continuously along a same direction or if the cell is simply oscillating back and forth. The asterisks in the above figures show data points with no statistical difference.

3.3 Cell Morphology

In order to validate that the migrational differences shown resulted from cells sensing a change in modulus and not a change in the created substrates SEM images of the PDMS-fiber interface and the cell morphology along the substrates were taken. These images can be seen in figure 2 below.

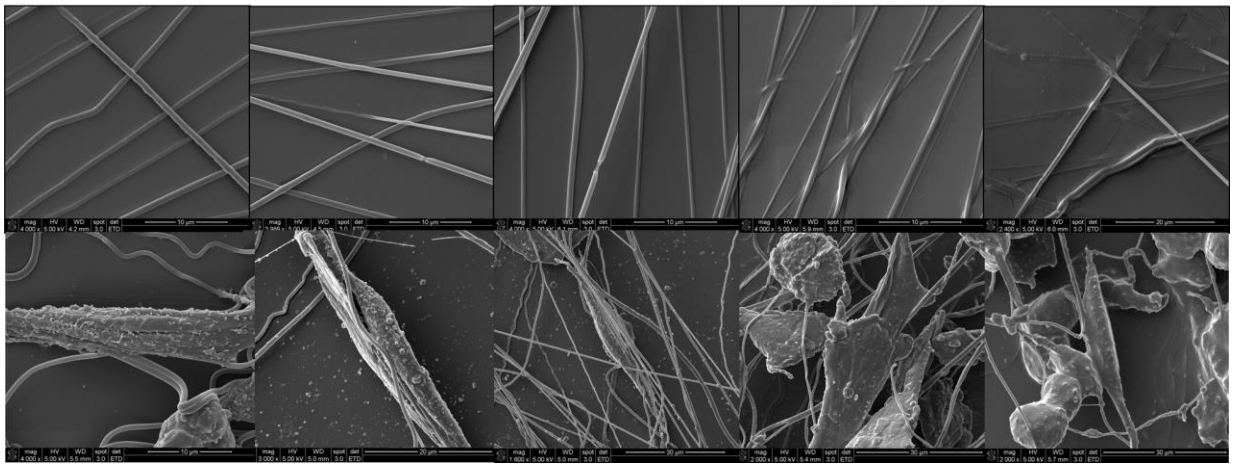

Figure 2:Top Row: Fiber-PDMS interface. Bottom Row: Cell Morphology along fiber. Left to Right: 100% 184, 80% 184, 20% 527, 50% 184, 50% 527, 20% 184 80% 527, 10% 184 90% 527.

The SEM images of the PDMS-fiber interface shows that as the substrate gets increasingly soft the fibers sink into the PDMS to an increasing extent. This supports the idea that the underlying PDMS modulus is felt by the cells.

The cell morphology images show cells elongated along the PCL nanofibers in all instances. This also supports the idea that the migration occurs through the same mechanism in all substrates and that migration is affected by the underlying modulus. The cell morphology is also

interesting to analyzed because images show cells extremely elongated in the 100% 185 sample which the 184 90% samples still show elongation just not to the degree that the 100% 184 cells do. The amount of elongation of each cell correlated to the migration.

4 Future Directions

4.1 Genetic Mechanism Determination

Future directions will involve experiments aimed at directly investigating the mechanism of cell migration along nanofiber alone. Intracellular actin stress fiber formation will be analyzed using immunofluorescence in order to locate a potential cause of the migrational differences. Focal adhesion complexes will also be analyzed using immunofluorescence to determine where the focal adhesion complexes are located. We seek to answer the important question: are the cells migrating and adhering solely to the PCL nanofibers or is there direct physical contact with the substrate? Genetic analysis will also be performed using western blotting techniques to determine possible differences in genetic mechanisms responsible for a change in migration as modulus changes.

4.2 Suspended Fibers

Suspended PCL nanofibers will also be formulated to determine how a cell migrates without the influence of any underlying substrate. This will be done using a 3-D printed mold as seen in figure 4.

Figure 4: 3-D Printed mold for the formation of PDMS ridged substrates.

The mold will be filled with Sylgard 186 PDMS and cure for 12-24 hours at 67°C. The PDMS will then be removed from the mold to create a ridged substrate. The substrates will be coated with aligned electrospun PCL nanofibers. MDA-MB-231 GFP labeled cells will be seeded onto the substrates and their migration potential analyzed. Thess substrates will give us an idea of the mechanism of how the cell is migrating. It will tell if the cell is using the underlying PDMS to

assist with migration or if migration is occurring solely by the cells adhering to the PCL nanofibers.

4. Conclusions

This study analyzed how changes are substrate modulus and topography affect cell migration. This was accomplished using a novel PDMS-fiber scaffold as a template for migration. These substrates were able to model tissue stiffness similar to that of the natural breast, tumor tissue, and stiffness far above these values. A general trend was discovered that showed an increased modulus causes an increase in total migration, net migration, and velocity. The study also analyzed how the morphology of a cell changes based on a change in topography and modulus. The future directions of this paper will evaluate possible genetic causes of migration changes and possible differences in stress fiber formation. Future experiments will also be performed to establish the effect of migration on suspended nanofibers alone without any possible direct physical influence of the underlying substrate.

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